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RNA from the yeast transposable element Ty*1* has both ends in the direct repeats, a structure similar to retrovirus RNA

(*Saccharomyces cerevisiae*/repetitive DNA/transcription/DNA sequence)

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ABSTRACT **The RNA homologous to the yeast transposable element Ty*1* is one of the more abundant poly(A)$^+$ RNAs in many strains of the yeast *Saccharomyces cerevisiae*. The 5′ and 3′ ends of Ty*1* RNA have been determined from analysis of cDNA. The 5′ end is 245 bases into the left δ sequence measured from the left side of the Ty*1* element. The δ sequence is a direct repeat of about 340 base pairs present at each end of the Ty*1* element. The Ty*1* transcription includes 93–97 bases of the left δ sequence and continues through the entire internal portion of the element and through about 295 bases of the right δ sequence before reaching the 3′ end located 38–46 bases from the right side of the right δ sequence. Because the δ sequences present at each end of a single Ty*1* element have identical or very similar DNA sequences, these end points for Ty*1* RNA raise several questions about the expression of Ty*1* elements. First, what are the initiation and termination signals, because the Ty*1* transcript must read through a DNA sequence that is identical to the 3′ end at about 50 bases from the 5′ end? Second, why is the direction of transcription of the Ty*1* element opposite to that of genes that are overexpressed after the insertion of a Ty*1* element? Third, because the Ty*1* RNA itself has direct repeats of about 45 bases, a structure analogous to retrovirus RNAs, is the Ty*1* RNA an intermediate in the transposition of Ty*1*?**

Transposable elements have recently been found in *Drosophila melanogaster* and the yeast *Saccharomyces cerevisiae* (1, 2). Although only the Ty*1* family of such elements has been well characterized in yeast, many different families of such elements occur in *Drosophila* (2, 3). Many of these elements share a basic structure of two direct repeats of a few hundred base pairs (bp) flanking an internal region of several kilobases (kb) of DNA (1–6). These transposable elements generate a small, direct repeat of 4–11 bp at the site of transposition (4, 5, 7), a property shared with bacterial transposons (summarized in ref. 8) and integrated proviruses of vertebrate retroviruses (9, 10). The exact number of base pairs generated is specific for the transposable element. For example, 5 bp are always generated by the yeast element Ty*1* and the *Drosophila* element *copia* (4, 5, 7). Another common characteristic of these elements is that they are heavily transcribed to give abundant poly(A)$^+$ RNAs (2, 11).

In addition to the abundant transcription of the element itself, Ty*1* can affect the expression of a gene flanking the element. Insertions of Ty*1* that inactivate the *his4* gene have been studied thoroughly by Fink and co-workers (4, 12–14). Another class of mutations produces 3–50 times more of the gene product after the insertion of a Ty*1* element near the 5′ end of the gene. Such Ty*1* insertion mutations that increase expression have been isolated for *CYC7*(15–17), *ADR2* (18), *HIS3* (19), *CAR1* (20), *DUR1* (21), and probably *CAR2* (16). The insertion of a Ty*1* element is then able to increase the expression of the flanking gene for many different genes.

Ty*1* RNA and the overexpression of a gene flanking a Ty*1* element show an unexpected relationship to the mating type of the yeast cell. In stationary *a* or α mating type cells, 5–10% of the poly(A)$^+$ RNA is Ty*1* RNA, but in *a*/α cells, the amount of Ty*1* RNA is 95% lower (11). Ty*1* transposition mutations that overexpress the flanking gene show a similar dependence on the mating type of the cell because the amount of gene expression is reduced 75–95% in *a*/α cells compared to that in *a* or α cells (16). The Ty*1* element then contains the necessary signals to place both its own expression and that of the flanking gene, in certain Ty*1* insertions, under mating-type control.

As a first step in understanding how this transcription and regulation occurs, the Ty*1* RNA is further characterized here. Previous work has shown that two sizes of Ty*1* RNA are present: the length of the most abundant one is 5.7 kb and of the less abundant one is 5.0 kb. The 5.7-kb RNA, which is only slightly smaller than the 5.9 kilobase pairs (kbp) of the Ty*1* element itself, contains at least some of the δ sequence, the direct repeat of about 340 bp present at each end of the Ty*1* element (11). Here it is shown that both the 5′ and 3′ ends of the 5.7-kb RNA are in the δ sequences. These end points raise a number of questions about the location and function of the transcription signals in the Ty*1* element; furthermore, they suggest that the Ty*1* RNA might be an intermediate in the transposition of the Ty*1* element because of its structural analogy to retrovirus RNAs.

MATERIALS AND METHODS

R-Loop Hybridization. Total nucleic acid was prepared by mixing yeast cells in a buffer/phenol/$CHCl_3$ mixture with glass beads. About 2×10^8 yeast cells in 1.5-ml tubes were centrifuged for 10 sec in a Microfuge. After the cells were washed with 1 ml of H_2O, they were resuspended in 0.5 ml of 0.5 M NaCl/0.2 M Tris, pH 7.5/0.01 M Na_3EDTA/1% $NaDodSO_4$. After 0.4 g of 0.45- to 0.50-mm glass beads and 0.5 ml of phenol/$CHCl_3$, 1:1 (vol/vol), were added, the tube contents were vigorously mixed for 2.5 min by taping the tubes to the head of a Vortex mixer. After centrifugation for 0.5 min in a Microfuge to separate the phases, the aqueous phase was removed and reextracted with 0.3 ml of phenol/$CHCl_3$. The aqueous phase was recovered from this extraction and placed in a new 1.5-ml tube. The tube was filled with ethanol containing 0.05% diethyl pyrocarbonate. After 0.5 hr or longer at −20°C, the total yeast nucleic acid was recovered by centrifuging for 1 min in a Microfuge. After the pellet was washed with 0.5 ml of 70% ETOH, the pellet was dissolved in 100 μl of H_2O. The yield was about

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Abbreviations: kb, kilobase(s); kbp, kilobase pair(s); bp, base pairs; cTy(s), Ty*1* cDNA(s).

100 μg of nucleic acid per 10^8 cells. Poly(A)$^+$ RNA was prepared by chromatography on poly(U)-Sepharose (22).

NNλ152 is the λ590 vector (23) containing the prototype D15 Ty*1* element in the same orientation as shown in the restriction enzyme map in ref. 1. NNλ261 contains a different Ty*1* element inserted in the same orientation as in NNλ152. Purified λ phages were mixed together in approximately equal amounts and denatured by NaOH as in the standard heteroduplex method (24). After neutralization, 15 μg of yeast poly(A)$^+$ RNA from stationary phase cells of the YNN5 yeast strain, also called X2180-1B, was added, and the nucleic acid was precipitated by addition of 2 vol of ethanol containing 0.01% diethyl pyrocarbonate. The pellet was taken up in 25 μl of 80% formamide/0.4 M NaCl/0.04 M Pipes, pH 7.0/1 mM Na_3 EDTA and incubated at 48°C for 3.8 hr and then at 37°C for 10 min. Treatment with glyoxal and the separation of the DNA from the RNA was carried out as described (25). Samples for electron microscopy were spread from 70% formamide/50 mM Tris/5 mM EDTA (pH 8.5)/0.05 mg of cytochrome *c* per ml over 40% formamide in distilled H_2O (26). The right arm of the λ590 phage was used as a length standard of 10.88 kbp (23, 27). The error limits given for the electron microscopy measurements are the standard error of the mean.

Sequence Determination of Ty*1* cDNA. The yeast cDNA pool in plasmid vector pMB9 was made by T. St. John using standard methods (28) with RNA from the YNN13 yeast strain, also called D585-11c. Clones containing Ty*1* cDNA were isolated by colony hybridization (29) with nick-translated (30) phage M13m p73–S13 [^{32}P]DNA in which the only yeast DNA is Ty*1* DNA (1, 31). Sequence determination of one strand of each cDNA was carried out by labeling each cDNA at the *Xho* I site in the plasmid by filling in with [^{32}P]dCTP and unlabeled dTTP (32). The sequences of the separated end-labeled fragments were determined by the method of Maxam and Gilbert (32). The primer extension experiment was carried out as described (33).

RESULTS

R-Loop Measurements. The ends of Ty*1* RNA were first approximately determined by an R-loop hybridization experiment. A heteroduplex between two recombinant λ phages containing Ty*1* elements with different flanking DNA sequences was hybridized with poly(A)$^+$ RNA to form R loops. The ends of the R loop could then be measured relative to the ends of the Ty*1* element defined by the heteroduplex. Fig. 1 outlines these hybridization experiments and presents measurements of 17 molecules. In all cases, the R loop was continuous with no evidence for intervening sequences. Length *a* and length *b* in Fig. 1 represent the distances into the Ty*1* element before the end of the Ty*1* RNA. Length *b*, which the results below will show is the 5′ end of the Ty*1* RNA, was 260 ± 40 bp long and constituted a single, homogeneous length class. Because the length of the δ sequence, the direct repeat present at each end of the Ty*1* element, is 331–338 bp (4, 5, 12), this end is 80 ± 40 bp into the δ sequence. Length *a*, which is the 3′ end, had two length classes of equal frequency, one of 20 ± 10 bp, a location in the δ sequence, and one of 630 ± 60 bp, a position in the internal part of the Ty*1* element rather than the δ sequence. As an internal control, the length of the δ sequence was measured as described in Fig. 1. The length of the δ sequence was measured to be 320 ± 20 bp, which agrees with the length of 331–338 bp determined by DNA sequence assay. This agreement indicates that the length measurements were accurate and that no significant separation of homologous DNA strands had occurred near the ends of the duplex region. From the R-loop measurements and the 5.9-kb length of the Ty*1* element, one expects RNAs of 5.6 and 5.0 kb, in good agreement with the lengths of 5.7 and 5.0 kb determined by hybridization of an RNA blot with Ty*1* probe (11). These measurements then indicate that both ends of the 5.7-kb Ty*1* RNA are in the δ sequences of the Ty*1* element.

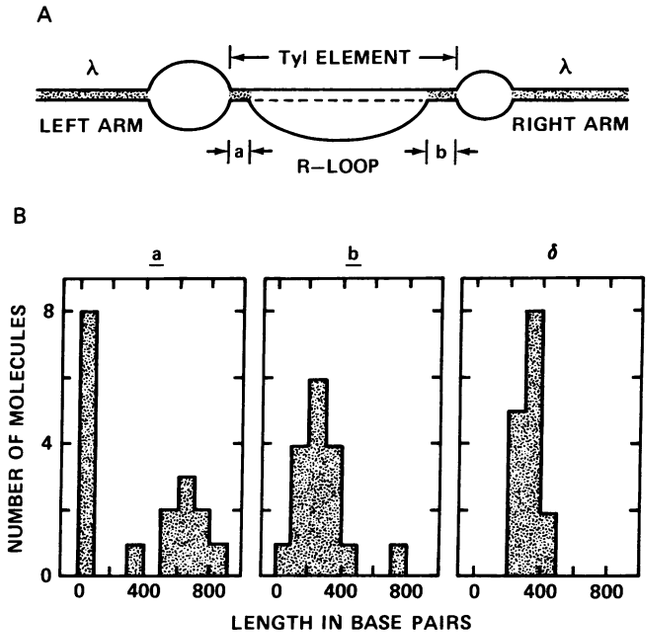

FIG. 1. R-loop hybridization to heteroduplex of Ty*1* elements. (*A*) The procedure used to determine the end points of Ty*1* RNA is outlined. A heteroduplex formed between two recombinant phages containing different Ty*1* elements in the same orientation gives a duplex region corresponding to the Ty*1* element itself surrounded immediately at each end by single-strand loops of nonhomologous DNA. After R-loop formation, lengths *a* and *b* were measured. The measurements of the δ-sequence length were made on heteroduplexes between λ phages that contained a δ-to-δ deletion. A recombinant λ phage containing a Ty*1* element frequently deletes one δ sequence and the internal part of the element by homologous recombination across the δ sequences (unpublished data). Heteroduplexes formed from the deleted forms of each λ phage have the δ sequence as a duplex between the two regions of nonhomologous flanking DNA. (*B*) Measurements of length *a*, length *b*, and the length of the δ sequence. The means of the two classes of lengths are *a* = 0.02 ± 0.01 and 0.63 ± 0.06 kb, *b* = 0.26 ± 0.04 kb, and δ = 0.32 ± 0.02 kb.

Sequence of Ty*1* cDNAs (cTys). The DNA sequences of cDNAs to Ty*1* RNA are shown in Fig. 2. The poly(A) tract should represent the 3′ end of the RNA at which poly(A) was originally present. For comparison, the DNA sequences of four different δ sequences (from DNA fragments designated 912, 917R, 917L, and D15) are included. Up to the poly(A) tract, cTy 1 and cTy 12 have sequences identical to the D15 Ty*1* δ sequence (5). However, cTys 1 and 12 differ at only two bases exactly at the 3′ end, which may indicate that the 3′ end point of the RNA is slightly heterogeneous. The sequences from cTys 3, 5, and 9 are similar but not identical to the δ sequences of the Ty*1* elements of 912 and 917 (4, 12). The differences between cTys 3, 5, and 9 also occur near the 3′ end. For example, cTys 3, 5, and 9 are identical for 108 nucleotides from position 56 to 164, while three differences between cTy 3 and cTy 5 and five differences between cTy 3 and cTy 9 occur in the last 13 nucleotides before the poly(A) tract. Therefore, all five cDNA sequences differ from one another. Because the DNA sequence of the δ sequences differs from element to element (4, 5, 12), this heterogeneity of cDNA sequences indicates that many Ty*1* elements are heavily transcribed to give approximately equal

```
912    T-  -  T-    --  T- -   GA T G        TGC       C    AT        A     T
917R   T-  -  T-    --  T- -   GA T G        TGC       C    AT        A
917L   T-  -  T-    --  T- -   GA T G        TGC       C    AT        A
          160         150        140       130       120       110       100
D15    CGGAATGAGGAATA--ATCGTAATATTAGTATGTAGAAATAT-AGATTCCATTTTGAGGATTCCTATATCCTCG
cTy#1                --                      -
cTy#3  T-  -  T-    AT  T- -   GA T G      - TGC       C    AT        A
cTy#5  C-  -  T-    AT  T- -   GA T G      - TGC       C    AT        A
cTy#9  T-  -  T-    AT  T- -   GA T G      - TGC       C    AT        A
cTy#12               --                      -


912                                                     -
917R                      -  AC             T   -     T-  A
917L                   C     AC             T   -     T-  A
          90         80         70        60        50        40        30
D15    AGGAGAACTTCTAGTATATTCTGTATACCTAATATTATAGCCTTTATC-AACAATGGAATCCCAACAATTA
cTy#1                                                   -          AAAAAAAAAAAA
cTy#3                     -  AC             T   -     T-  AAAAAAAAAAAAAAAAAAAA
cTy#5                     -  AC                 -      C  AAAAAAAAAAAAAAAAAAAA
cTy#9                     -  AC                    A   - CAAAAAAAAAAAAAAAAAAA
cTy#12                                                  -       AAAAAAAAAAAAAAA
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FIG. 2. Sequence of cTys near the poly(A) tract. The sequence of the D15 δ DNA (5) is written out. Above the D15 line, the DNA sequence of three other δ sequences (4, 12) are shown only where they differ from the D15 sequence. A hyphen indicates no nucleotide is present at that position. Similarly, the five cDNA sequences are given below the D15 line.

fractions of the large amount of the total Ty*1* RNA present in the yeast cell.

If one counts from the first G, C, or T adjacent to the run of As in the cDNA sequences shown in Fig. 2, the 3′ end varies from 38–46 bp before the end of the δ sequence and the Ty*1* element. This agrees with the end point of 20 ± 10 bp determined from the R-loop measurements and identifies this as the 3′ end of the 5.7-kb Ty*1* RNA.

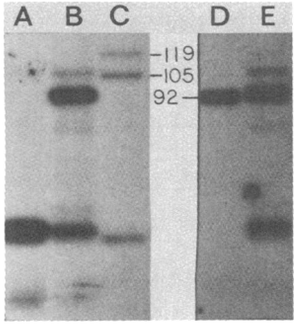

FIG. 3. Primer extension experiment to determine the 5′ end of Ty*1* RNA. The primer fragment was made by 5′-end labeling with [γ-^{32}P]-ATP and polynucleotide kinase at the left *Pvu* II site (P_1) in the D15 Ty*1* element shown in Fig. 4. After *Hpa* I digestion, the 0.97 kb *Hpa* I–*Pvu* II fragment (H_1–P_1) labeled only at the *Pvu* II end was isolated. Digestion with *Hin*fI generated a *Hin*fI–*Pvu* II fragment about 170 bp long that was used as the primer fragment. About 3 ng of this primer fragment was hybridized to 16 μg of poly(A)$^+$ RNA from stationary-phase cells of the YNN4 yeast strain, also called X2180-1A. After incubation with reverse transcriptase to extend the primer hybridized to Ty*1* RNA, the samples were denatured and run on a 6% acrylamide/urea sequence determination gel (32). Lanes: A, primer fragment carried through the procedure with no poly(A)$^+$ RNA added to the hybridization reaction; B, extended primer reaction; C, size standards generated by partial *Hin*fI digest of the 0.97-kb end-labeled fragment [from the DNA sequence (5), *Hin*fI sites occurred at positions 115, 105, and 42 bp into the δ sequence]; D, size standard generated by *Xho* I digestion of the 0.97-kb end-labeled fragment [*Xho* I site is at position 92 in the δ sequence (5)]; E, mixture of extended primer fragment and length standards. The extended primer fragment and the *Xho* I-digested fragment are partially resolved. The minor band of the extended primer is about 107 bases into the δ sequence, whereas the major band is 95 ± 2 bases into the δ sequence. The error limits on the size of the extended primer were estimated from tracks done on a different end-labeled fragment run on the same gel (not shown). Discrete bands were present, although they were too closely spaced to read the sequence. Sizes are shown as bp into the δ sequence.

5′ End of Ty*1* RNA. Fig. 3 shows how the 5′ end was localized by a primer extension experiment. About 5% of the extended primer fragment corresponded to 107 bases into the δ sequence, but the major fraction containing about 95% of the extended primer was in a narrow band at a point 95 ± 2 bases into the δ sequence (measured from the right side of the left δ sequence). This agrees with the R-loop measurement of 80 ± 40 bases.

DISCUSSION

The end points determined here show that the 5′ end of the 5.7-kb Ty*1* RNA is 95 ± 2 bases into the δ sequence from the internal part of Ty*1*. Transcription continues through the 95 bp of δ sequence, through all of the internal part of the Ty*1* element, and through about 295 bp of the δ sequence at the opposite end before the 3′ end occurs about 40 bp before the end of the δ sequence and the Ty*1* element. Preliminary experiments indicate that the less abundant 5.0-kb Ty*1* RNA is transcribed from the same strand as the 5.7-kb RNA. From the R-loop measurements, therefore, the 5.0-kb RNA has the same 5′ end as does the 5.7-kb RNA but has a 3′ end in the internal portion of Ty*1*.

Although the exact ends of *copia* RNA from the *Drosophila copia* element have not been published, it seems likely that *copia* RNA is similar to Ty*1* RNA. The size of the *copia* RNA is 5.2 kb compared to 5.0 kb for the element (34), and the RNA is known to contain sequences homologous to the direct repeats present at each end of the *copia* element (35). The RNA from *copia*, and perhaps from similar *Drosophila* elements, also may have both the 5′ and 3′ ends in the direct repeats present at each end of the element.

The 5′ and 3′ end points of the 5.7-kb Ty*1* RNA raise a number of interesting questions about the transcriptional signals of the Ty*1* element. For example, an identical or very nearly iden-

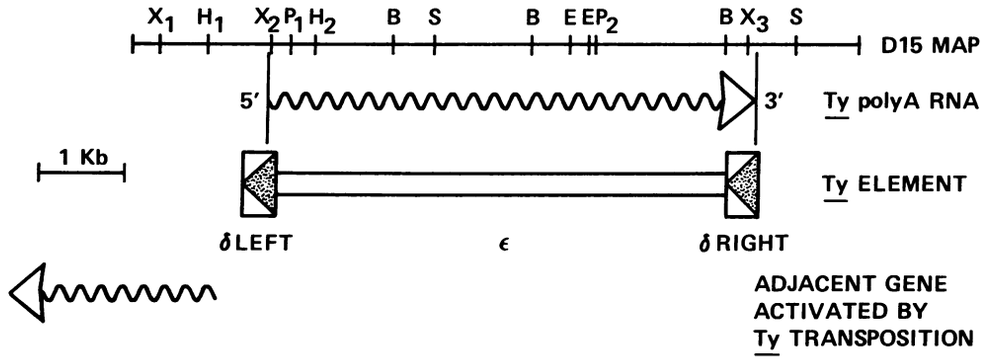

FIG. 4. Ty*1* RNA end points and orientation. The straight line represents the D15 fragment containing a Ty*1* element plus flanking yeast DNA. The wavy line below this represents the 5.7-kb Ty*1* RNA. Stippled triangles represent the δ sequences, which are at each end of the Ty*1* element (▭). The restriction sites for the D15 fragment are from ref. 1 and unpublished data. Based on the restriction sites in the internal part of the Ty*1* element, transcription of the Ty*1* element and of the gene overexpressed after insertion of a Ty*1* element are in opposite directions (16). X, *Xho* I; H, *Hpa* I; P, *Pvu* II; B, *Bgl* II; S, *Sal* I; E, *Eco*RI.

tical DNA sequence is present both at the 3′ end and about 50 bp beyond the 5′ end of the RNA. The transcription must continue beyond this sequence at the 5′ end at a reasonably high frequency to give the large amount of Ty*1* RNA in the yeast cell. Another question is the location of the promoter for Ty*1* RNA. The finding that many Ty*1* elements are expressed suggests that the Ty*1* element carries its own promoter because the DNA flanking the elements is generally different for each specific Ty*1* element. The most likely location for the promoter is the 240 bp of the δ sequence 5′ to the 5′ end of the RNA, but this sequence is repeated in the opposite δ sequence. It is not known whether the sequence initiates a transcript in this opposite δ sequence that either terminates after 50 bp or reads out of the δ sequence into DNA flanking the Ty*1* element.

This hypothetical transcript reading out of the Ty*1* element cannot explain the ability of the Ty*1* element to increase the expression of a flanking gene because the Ty*1* is always in the opposite orientation in these mutations. That is, the Ty*1* element and the gene overproduced are transcribed in opposite directions. There are more than 10 cases for which the orientation of the Ty*1* element is known [*CYC7*, two independent Ty*1* insertions (16, 17); *HIS3*, six independent Ty*1* insertions (19); *ADR2*, seven independent insertions (18)]. No example of increased gene expression with Ty*1* in the opposite orientation has been characterized, so it seems likely that the intact Ty*1* element can increase gene expression in only this one orientation. However, the Ty*1* element in the *his4*–917 mutation, which inactivates the *HIS4* gene, is in the same orientation (12) as these insertions that increase gene expression, so more than just the presence of the Ty*1* element in this orientation is required to overexpress the flanking gene.

The end points of Ty*1* RNA also suggest that Ty*1* RNA might be an intermediate in the transposition of Ty*1* RNA. Because of the δ sequence of the element, the 5.7-kb Ty*1* RNA contains all of the sequence information of the element and is then a potential intermediate in the transposition process. The principal reason for taking this possibility seriously is the strong structural similarities between Ty*1* and retroviruses, some of which have been pointed out earlier (36). Another structural similarity between Ty*1* and retroviruses is shown here in that the 5.7-kb Ty*1* RNA itself has direct repeats of about 45 bases similar to the direct repeats of retrovirus RNAs, about 20 bases in the case of avian myeloblastosis and Rous sarcoma retroviruses (37–39) and 60 bases for Moloney murine leukemia retrovirus (40). Because the direct repeats on the retrovirus RNA are used to jump from one end of the RNA to the other during cDNA synthesis (41), the presence of direct repeats suggests that Ty*1* RNA might also be made into a DNA copy.

One further intriguing structural analogy between retroviruses and Ty*1* is the transfer RNA bound to the RNA. In retrovirus RNA, a transfer RNA is bound near the 5′ end of the RNA just inside of the direct repeat. The tRNA, which is specific for the retrovirus, serves as the primer for DNA synthesis (41). Eibel *et al.* (36) have pointed out that $trNA_I^{Met}$ of yeast contains a region homologous to the Ty*1* element just inside of the δ sequence. With the end points and orientation of the Ty*1* RNA determined here, one finds that this homology between Ty*1* RNA and $tRNA_I^{Met}$ occurs near the 5′ end of the Ty*1* RNA just inside of the δ sequence, a location exactly analogous to that of tRNA primers on retrovirus RNAs. In light of these structural similarities, it seems likely that Ty*1*, *copia*, other similar *Drosophila* elements, and retroviruses have functional and mechanistic similarities as well.

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